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Wood-Degrading Fungi as Degraders of Hazardous Waste

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by

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The use of fungi to degrade waste materials has not been investigated to any extent until recently. From the perspective of sewage treatment, filamentous fungi were to be avoided due to processing problems and in some cases fungi found in sewage can be pathogenic. Based on these considerations, it may come as some surprise that a wood rotting fungi is now being investigated as a candidate organism for the treatment of hazardous waste.

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The biological detoxification of hazardous waste is largely an underdeveloped technology. Bacterial species are known to possess a variety of detoxification skills, apparently associated with the need to survive. Single bacterial species may not have the ability to convert a toxicant to carbon dioxide and water. With the use of bacterial communities, there is the feature that each species of the consortium plays a role in the overall degradation of the waste with the inadequacies of one covered by the abilities of another. The stability of such consortia is of some concern since there may be environmental effects that permit the selection of certain members over others resulting in the loss or slowing of the degradation process.

The use of fungi to degrade waste materials has not been investigated to any extent until recently. From the perspective of sewage treatment, filamentous fungi were to be avoided due to processing problems and in some cases fungi found in sewage can be pathogenic. Based on these considerations, it may come as some surprise that a wood rotting fungi is now being investigated as a candidate organism for the treatment of hazardous waste.

Wood-degrading fungi are divided into three main categories: soft, white and brown rotting fungi. The brown or white characterization is based on the residue left after the fungus has infested a suitable host. The brown coloration, characteristic of a brown rot, wood rotting fungus is attributable to portions of lignin incompletely degraded by the fungus and is to be contrasted with the white rot fungi that have more complete capabilities to degrade lignin.

Phanerochaete chrysosporium is a filamentous, white, wood rotting fungus and has been typed to be a member of the Hymenomycetes subclass of Basidiomycetes. Fungi are eukaryotic, i.e., they possess a nuclear membrane and as microorganisms are considered to be plantlike without chlorophyll having no photosynthetic abilities.

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White rot fungi are primary wood degraders in nature (1). All components of wood are degraded by these fungi with the naturally occurring polymers of cellulose and lignin forming the major sources of carbon to assist fungal growth. Of the two general polymers, lignin, a structural component of wood, is by far the more difficult to degrade due to its composition as a heteropolymer formed from the cross linking of three precursor cinnamyl alcohols (2). Of necessity, the fungus must be able to switch its ability to degrade these various polymers as the concentration of polymer varies with the composition of the wood. This ability for P. chrysosporium is controlled by the absence of certain nutrients. Nitrogen deficiency is generally used to induce this secondary metabolic cycle of lignin utilization.

Degradation studies demonstrated that the white rot fungus can degrade a variety of structurally diverse compounds (3,4). The following generalizations regarding biodegradability as affected by structural features of the compounds have been made: 1) chlorine substituents inhibit mineralization but do not prevent it, 2) mineralization of chlorinated aromatic compounds apparently requires the presence of a substituent other than chlorine, and 3) the fungus is capable of mineralizing chlorinated aliphatic compounds (5).

The lignin degrading system of P. chrysosporium was confirmed to be at least partly responsible for the biodegradation that occurred. The disappearance of ^{14}C-PCP and ^{14}C-DDT was similar to those obtained for ^{14}C-lignin mineralization. Substrate disappearance, metabolite identification, mass balance analysis, and ^{14}C-DDT mineralization clearly demonstrated the capability of P. chrysosporium to treat DDT wastes (6). A proposed fungal metabolic pathway different from the major pathway proposed for the major bacterial degradation of DDT occurring in the environment was formulated in this study. Other fungi, Pleurotus ostreatus, Phellinus weirii, and Polyporus versicolor, were also found to mineralize DDT.

The ability of the fungal extracellular enzymes to oxidize polynuclear hydrocarbons has been related to the ionization potential of these compounds (7). When pyrene is used as a substrate with H8 both pyrene-1,6-dione and pyrene-1,8-dione are the major products. In similar fashion, anthracene is converted to antraquinone and benz(a)anthracene yields 7,12- benz(a)anthrquinone (7). The initial quinone metabolites, pyrene-1,6-dione and pyrene-1,8-dione, are mutagenic by the Ames test. When cultures of the fungus are innoculated with the starting hydrocarbon substrate these diones do not accumulate.

Dibenzodioxin and 2-chlorodibenzodioxin have been found to be oxidized by one of the fungal extracellular ligninases in the presence of hydrogen peroxide (7). Chlorinated phenols have been found to be very suitable substrates, and product identification is under way (8,9,10,11).

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A water treatment process developed jointly by North Carolina State University and the USDA Forest Products Laboratory will be investigated in the next year with a laboratory bench-sized unit and scaled up to determine its treatment efficacy. Based on the organism's ability to degrade polynuclear aromatic compounds, the first application of this technology will be the treatment of waste derived from wood treating waste sites. In the United States these sites are a major problem awaiting cleanup. The total number of sites exceeds 700. The patented reactor (12) is a specially designed rotating biological contractor that utilizes P. chrysosporium as the biological species for treatment. Since the fungus doesn't have the same adhesion to smooth surfaces as do bacterial species, the reactor design is modified to permit attachment of the mycelial mass to the plates. Recent results derived from the bench scale operation of this technology show that this reactor will degrade 250 ppm pentachlorophenol in water to 5 ppm in 8 hrs. (13). Pink water associated with munitions production is adequately treated. Degradation of 2,4,6 trinitrotoluene and 2,4-dinitrotoluene are degraded in concentrations up to 150 ppm in 24 hrs. (13). In both cases several sequential doses of the original concentration of contaminant are removed with the same degree of ability. These results point to the interpretation that treatment is occurring and not merely absorption onto the mycelial mat. Plans to scale this technology in the next twelve months include pilot-scale operations to treat surrogate waste and once operational conditions are established leachate derived from an actual wood treating site will be treated. Preliminary designs for this treatment at full scale call for parallel treatment trains.

The general success of solution biodegradation studies with the fungus stimulated speculation that this microorganism may be an appropriate candidate for the treatment of contaminated soils (14,15). The focus of application for soil treatment of the fungus is the remediation of wood treating sites. Recent research has assessed the effects of selected soil types, temperatures, pH, and water potentials on the growth of the fungus in sterile and non-sterile soils (16,17). Three well characterized soils (topsoil and subsoil) were used in this work. Biomass accumulations as well as growth habit of P. chrysosporium were greatly influenced by soil type. soil nitrogen content appears to be the primary factor responsible for differences in fungal growth in the three studied soils. Growth was strongly and positively correlated with nitrogen content. This factor, therefore, appears to play a major role in mediating the growth of the fungus in soil, and is easily controlled by nitrogen supplementation.

The application of the fungus to treat contaminated soils will include small scale treatment of selected pollutants at environmentally significant concentrations, the evaluation of amendments on primary and secondary metabolism, and the delivery of oxygen within the soil to the growing fungus. Ancillary investigations will look into the development of better estimates of fungal biomass in soil, the importance of

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soil sterilization to growth of the fungus, and inoculum development.

The white rot fungus research continues to stimulate interest and shows promise for field applications. Parts of the research program sponsored by the Risk Reduction Engineering Laboratory are starting to develop major advances in the emerging biological control technology field.

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